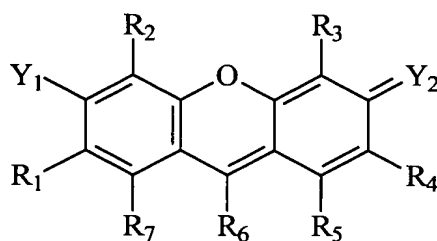


ABSTRACT

A class of aromatic-substituted xanthene compounds useful as fluorescent dyes is disclosed, the compounds having the general structure



where Y_1 and Y_2 taken separately are selected from the group consisting of hydroxyl, oxygen, imminium, linking group and amine, or Y_1 taken together with R_2 is cyclic imine, or Y_2 taken together with R_3 is cyclic amine; R_2 , R_3 , R_5 , and R_7 taken separately are selected from the group consisting of hydrogen, fluorine, chlorine, lower alkyl, lower alkene, lower alkyne, sulfonate, sulfone, amino, imminium, amido, nitrile, lower alkoxy, phenyl, and linking group; R_1 taken separately is selected from the group consisting of phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic, linking group and electron-rich heterocycle, or when taken together with R_7 is selected from the group consisting of electron-rich heterocycle and indene; R_4 taken separately is selected from the group consisting of amino, amido, phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic, indene, linking group and electron-rich heterocycle, or when taken together with R_5 is selected from the group consisting of phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic, indene, and electron-rich heterocycle; and R_6 is selected from the group consisting of acetylene, lower alkyl, lower alkene, cyano, phenyl, substituted phenyl, and heterocyclic aromatic. In another aspect, the invention includes methods for synthesizing the above dye compounds and intermediates. In yet another aspect, the present invention includes reagents labeled with the asymmetric benzoxanthene dye compounds, including deoxynucleotides, dideoxynucleotides, phosphoramidites, and polynucleotides. In an additional aspect, the invention includes methods utilizing such dye compounds and reagents including dideoxy polynucleotide sequencing and fragment analysis methods.